Stimulation of Macrophages by Retinal Proteins: Production of Reactive Nitrogen and Oxygen Metabolites

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PURPOSE. In previous work, it has been shown that in experimental autoimmune uveitis, the peroxynitrite-mediated protein nitrination product nitrotyrosine was localized in the degenerating photoreceptors. Subsequently, phagocyte-generated inducible nitric oxide synthase (iNOS) was also found to localize, primarily in the outer retina and to a lesser extent in the anterior segments. This study was intended to determine whether retinal soluble proteins such as S-antigen and interphotoreceptor retinoid-binding protein (IRBP) play a role in the induction of NO and superoxide by a macrophage cell line and by rat and rabbit peritoneal macrophages.

METHODS. Cells from the murine macrophage cell line RAW 264.7 and rat and rabbit peritoneal macrophages were incubated in the presence of retinal soluble proteins. The nitrite level in the cultured supernatant was evaluated for NO production using the Griess reaction. Activation of nuclear transcription factor κB (NF-κB) was determined by electrophoretic mobility shift assay. Superoxide production was measured by superoxide dismutase-inhibitable reduction of cytochrome C.

RESULTS. Both S-antigen and IRBP induced significant, dose-dependent nitrite production in RAW 264.7 and rat peritoneal macrophages. Induction of iNOS by retinal proteins was inhibited by the iNOS-specific inhibitor aminoguanidine and the tyrosine kinase inhibitor genistein. This iNOS induction was accompanied by the activation of NF-κB. S-antigen also induced superoxide production in rabbit peritoneal macrophages, but not in RAW 264.7.

CONCLUSIONS. These results show that soluble retinal proteins significantly induce NO and superoxide production by macrophages. Increased production of reactive oxygen species by macrophages in the presence of these soluble retinal proteins in vivo may accelerate photoreceptor degeneration in uveitis. (Invest Ophthalmol Vis Sci. 1999;40:3215–3223)

Nitric oxide (NO) is a free radical molecule formed from tissue L-arginine. This conversion is catalyzed by nitric oxide synthase (NOS). Inducible nitric oxide synthase (iNOS) is the main form of NOS in bone marrow–derived macrophages. Although both NO and superoxide (another oxidant released by the phagocyte) display low chemical reactivity, their facile combination yields the potent oxidizing-nitrating agent peroxynitrite. We have reported that peroxynitrite mediates damage to photoreceptors in experimental autoimmune uveitis (EAU), an animal model for the study of human uveitis.

We have also shown that in EAU, there is a selective expression of iNOS by the macrophages in the outer retina, but not in other affected ocular sites. Therefore, it appears there are local factors that play a role in this selective expression of iNOS in the retina. One local factor that is unique to the outer retina is the abundance of several soluble proteins, the most important being S-antigen and interphotoreceptor retinoid-binding protein (IRBP). However, the presence of factors that inhibit iNOS induction, such as transforming growth factor-β (TGF-β), at the anterior segment sites cannot be totally ruled out.

The fact that iNOS-positive staining is found in the outer retina and not in the anterior segments or the uveal tract in EAU prompted us to examine whether retinal proteins, such as S-antigen and IRBP, could serve as other enhancing factors in the induction and activity of iNOS and therefore in NO production of macrophages. In assessing the stimulatory capacity of these retinal proteins, it is also important to determine whether superoxide is induced simultaneously, because superoxide is a major oxygen metabolite released by the activated phagocytes.

Because S-antigen and IRBP are constitutively present in large amounts in the retina, the finding of substantial activation by these proteins could indicate an additive-synergistic effect of NO and superoxide production in these sites, thus accelerating phagocyte-mediated retinal damage in uveitis or other related intraocular inflammations.
MATERIALS AND METHODS

Animals and Reagents

Lewis rats, each weighing 150 to 175 g, were obtained from Charles River (Wilmington, MA). Pigmented rabbits, each weighing 1400 to 1800 g, were obtained from Irish Farms (Norco, CA). All animals used for the cell cultures were maintained and treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Brewer’s thioglycollate broth was purchased from Difco Laboratories (Detroit, MI). Dulbecco’s modified Eagle’s medium (DMEM), penicillin-streptomycin, and a nuclear factor (NF)-κB–binding protein detection system were purchased from Gibco (Grand Island, NY). Fetal bovine serum was purchased from Atlanta Biologicals (Norcross, GA). Bisindolylmaleimide I (GF 109203X) and 2′-amino-3′-methoxyflavone (PD 98059) were obtained from Calbiochem (San Diego, CA). Herbimycin A was obtained from Biomol (Plymouth Meeting, PA). [γ-32P]-adenosine triphosphate (ATP) was obtained from ICN Biomedicals (Irvine, CA). Hanks’ balanced salt solution (HBSS), glycerol, in a total volume of 25 μl, was incubated in the final purified S-antigen and IRBP were found to be 2.3% and 1.4% of the total soluble retinal proteins. These values were used for calculating the rat S-antigen and IRBP in the extract of soluble retinal proteins.

Preparation of S-Antigen, IRBP, and 15mer Peptides

S-antigen and IRBP were isolated from fresh bovine eyes, as previously described.5,6 The sequence of human retinal S-antigen has been published.7 S-antigen sequence 185-199 PLEMG-QPRAETWQ (peptide 1), which contains the 2-6-11 motif, was chemically synthesized by the solid-phase method8 with the Fmoc modification9 using a one-column peptide synthesizer (model 430 A; Applied Biosystems; Foster City, CA). Mutant peptide PTEMGGQPRAETWQ (peptide 2), which does not have the 2-6-11 motif, was synthesized by the same method. The crude rat retinal soluble proteins from 32 Lewis rat eyes were extracted using a procedure similar to that for extraction of bovine S-antigen.10 In the reported preparation, the final purified S-antigen and IRBP were found to be 2.3% and 1.4% of the total soluble retinal proteins. These values were used for calculating the rat S-antigen and IRBP in the extract of soluble retinal proteins.

Cell Culture

Murine macrophage cell line RAW 264.7 was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown at 37°C with 10% CO2 in DMEM supplemented with 10% fetal bovine serum and 100 μg/ml each of penicillin and streptomycin. Glycogen- or thiglycollate-elicited peritoneal macrophages were obtained from rats and rabbits and cultured as previously described.11 The peritoneal exudate macrophages were centrifuged at 1000 rpm for 5 minutes. When cells were contaminated by erythrocytes, pellets were suspended in ammonium chloride-potassium bicarbonate buffer for 5 minutes at room temperature and centrifuged at 1000 rpm for 5 minutes. Cells were resuspended in DMEM supplemented with 10% fetal bovine serum and 100 μg/ml each of penicillin and streptomycin and incubated for 2 hours at 37°C. Nonadherent cells were removed by aspiration. More than 95% of the adherent cells were macrophages, as shown by nonspecific esterase staining. The viability of macrophages, determined by trypan blue exclusion, was more than 95% of the total counts.

Nitrite Production

NO production in culture supernatants was measured as nitrite accumulation.12 Cells were collected and resuspended in DMEM without phenol red plus 10% fetal bovine serum and 100 μg/ml each of penicillin and streptomycin to a concentration of 1 × 106 cells/ml. Cells were plated at 1 ml/well in 24-well culture plates and allowed to adhere for 4 hours. Thereafter, the medium was replaced with fresh DMEM containing various agents. The supernatants were collected after the desired period of incubation, and 0.5 ml of the supernatant was incubated with the same amount of Griess reagent (0.1% N-(1-naphthyl)-ethylenediamine · 2HCl, sulfanilamide, pyrroldine dithiocarbamate (PDTC), dithiothreitol, Tris, bovine serum albumin (BSA), glycerol, superoxide dismutase (SOD), cytochrome C, myocin, N-formylmethionyl-leucyl-phenylalanine (FMLP), polymixin B, human recombinant tumor necrosis factor (TNF)-α, and human recombinant interferon (IFN)-γ were purchased from Sigma (St. Louis, MO). Bovine rhodopsin was a gift from Paul A. Hargrave (University of Florida); bovine recoverin was from James B. Hurley (University of Washington); bovine phosducin was from Cheryl M. Craft (University of Southern California); and S-antigen fragment peptides were from Dale S. Gregerson (University of Minnesota).

Electrophoretic Mobility Shift Assay for Transcription Factor NF-κB

Electrophoretic mobility shift assay for NF-κB was performed as previously described.14 Raw 264.7 cells (3 × 106 cells) were incubated at 37°C with 50 μg/ml Suntigen for times ranging from 30 to 240 minutes, in the presence or absence of pharmacologic inhibitors. At the end of the incubation period, cells were washed with phosphate-buffered saline (PBS), and nuclear extracts were prepared as previously described.15 Double-strand oligonucleotide (5 ng) containing a tandem repeat of the NF-κB DNA-binding sequence -GGGGACTTTCC- was end-labeled with 100 μCi [γ-32P]deoxyadenosine triphosphate (dATP) using T4 polynucleotide kinase as suggested in the manufacturer’s kit. The DNA-binding reaction mixture containing nuclear extract (5 μg protein), 10 mM Tris (pH 7.6), 50 mM NaCl, 1 mM dithiothreitol, 0.02 μM ATP, 5 μg BSA, and 10% glycerol, in a total volume of 25 μl, was incubated in the
Nitrite production by macrophage cell line RAW 264.7 cells (1 × 10⁶ cells) were incubated with 50 µg/ml S-antigen or IRBP at 37°C. Production of nitrite was determined by the Griess reaction. Data are means ± SD for three determinations at each time point.

presence or absence of excess unlabeled oligonucleotide. The mixture was preincubated on ice for 15 minutes, followed by the addition of 1 × 10⁶ cpm 32P-labeled probe, and the binding reaction was allowed to proceed for 20 minutes at room temperature. The samples were then subjected to electrophoresis on 6% nondenaturing polyacrylamide gels using 0.25% TBE running buffer (25 mM Tris [pH 8.0], 22.5 mM borate, and 0.025 mM EDTA) at 150 V for 2 to 3 hours. The gels were dried and exposed to x-ray film (X-Omat AR; Eastman Kodak, Rochester, NY) followed by autoradiography.

Superoxide Production

Generation of superoxide was measured by the SOD-inhibitable reduction of cytochrome C. Both discontinuous (fixed time) assay and continuous assay were used. Basic assay procedures were performed as follows: cells were collected and resuspended in HBSS. Two tubes of cell suspension (1 × 10⁶ cells) in HBSS, one with 10 µl SOD (3 mg/ml) and the other with 10 µl water, were incubated for 2 minutes at 37°C before the addition of 50 µl cytochrome C (30 mg/ml) plus various agents (50 µg/ml S-antigen, 50 µg/ml IRBP, 50 µg/ml MBP, and 0.5 µM fMLP). The mixture was then incubated at 37°C in a shaking water bath for times ranging from 15 to 60 minutes. The fixed time assay reaction was stopped by placing the tubes in ice, and cells were removed by centrifuging at 1500 rpm for 5 minutes. For the continuous assay, at different time points, the reaction mixture was centrifuged briefly (500 rpm for 2 minutes) and the supernatant removed for measurement. The reduced cytochrome C was measured in a double-beam spectrophotometer, scanning between 570 and 530 nm (maximum, 550 nm), using the SOD-containing sample as the reference. The amount of superoxide produced was calculated by the molar extinction coefficient 21,000/M/cm. The data collection was performed as described for nitrite production. Briefly, two to three duplicate tubes were assayed within the same experiment from the same batch of peritoneal cells, these numbers were averaged, and three averages from separate collection of peritoneal cells were used to calculate mean ± SD.

RESULTS

Nitrite Production

Using nonprimed RAW 264.7 without exogenous L-arginine, both 50 µg/ml S-antigen and 50 µg/ml IRBP substantially induced the production of NO, measured by the accumulation of nitrite in culture supernatants. Induction by both agents demonstrated a near linear increase for up to 48 hours. The linearity in production of nitrite was less prominent with both S-antigen and IRBP after 48 hours. Under the same conditions, stimulation by IRBP was approximately 30% less than that produced by S-antigen (Fig. 1). In RAW 264.7, the production of nitrite induced by these two agents followed a concentration-dependent pattern of increase. From 0.01 to 1 µg/ml, the formation of nitrite was minimal. Beyond this concentration range, however, production increased rapidly. Thereafter, the formation of nitrite was dose dependent up to 50 µg/ml; above this concentration, a plateau was observed (Fig. 2). As observed in time-dependent production (Fig. 1), IRBP also displayed a smaller amount of stimulation compared with that of S-antigen. The control antigens including MBP, BSA, and myocin with the same concentration range induced no detectable nitrite production (Fig. 2). Other uveitogenic antigens—rhodopsin, recoverin, and phosducin—in the concentration range of 50 µg/ml were found to cause no significant stimulatory effect, with the production of nitrite by 10⁶ RAW 264.7 cells being 0.12 ± 0.13, 0.94 ± 0.78, and 1.54 nanomoles, respectively.

The stimulation capacity of these two soluble retinal proteins was next compared with other molecules and stimulants in RAW 264.7. Nitrite generation by 50 µg/ml S-antigen was

FIGURE 1. Kinetics of retinal protein-induced nitrite production by macrophage cell line RAW 264.7. RAW 264.7 cells (1 × 10⁶ cells) were incubated with 50 µg/ml of S-antigen or IRBP at 37°C. Production of nitrite was determined by the Griess reaction. Data are means ± SD for three determinations at each time point.

FIGURE 2. Nitrite production by macrophage cell line RAW 264.7 as a function of retinal soluble protein concentration. RAW 264.7 cells (1 × 10⁶ cells) were incubated with various concentrations of S-antigen, IRBP, MBP, BSA, or myocin for 48 hours at 37°C. Production of nitrite was determined by the Griess reaction. Data are the means ± SD for three determinations.
comparable to that of the potent stimulant LPS. Both synthetic peptides (50 μg/ml), peptide 1 (with the 2-6-11 motif) and peptide 2 (without the 2-6-11 motif), produced low levels of activation. The amount of nitrite produced was only 6% of that produced by 50 μg/ml S-antigen, and no significant difference in production was detected between the two synthetic peptides. Under the same conditions, MBP, a nonrelevant protein that functions as an autoantigen for allergic encephalomyelitis, did not induce nitrite production (Fig. 3A). This same trend held when primary cultures of rat peritoneal macrophages were used in place of RAW 264.7. In this system, overall production was substantially lower than that of RAW 264.7. However, the nitrite produced by S-antigen (50 μg/ml) especially was comparable to 10 μg/ml LPS, a common concentration used in the evaluation of this system (Fig. 3B). It appeared that the stimulatory effects of bovine S-antigen and IRBP were not caused by the xenogenic effect on the tested macrophages, because the retinal soluble proteins (containing S-antigen and IRBP) from Lewis rats also produced a stimulatory effect when tested against peritoneal macrophages obtained from Lewis rats. The nitrite production obtained by the rat S-antigen and IRBP was 1.2 ± 0.38, 1.68 ± 0.61, 1.34 ± 0.27, 3.02 ± 1.28, and 5.49 ± 0.9 nanomoles/10⁶ cells for the concentrations of 0.37, 1.22, 3.70, 18.5, and 37.0 μg/ml, respectively. In search

**Figure 3.** Induction of nitrite production by various agents. (A) The macrophage cell line RAW 264.7 (1 × 10⁶ cells) was incubated with 50 μg/ml each of S-antigen, IRBP, synthetic peptides (peptide 1 and peptide 2), MBP or with 10 μg/ml LPS for 48 hours at 37°C. (B) Glycogen-elicited rat peritoneal macrophages (1 × 10⁶ cells) were incubated with 50 μg/ml of S-antigen, IRBP, synthetic peptides (peptide 1 and peptide 2), or MBP for 48 hours at 37°C. The incubation with 10 μg/ml LPS was also included. The data for control (cultures not receiving any agents) represent the background levels of nitrite. Production of nitrite was determined by the Griess reaction. Data are means ± SD for three determinations.
of active stimulatory sequence motif in S-antigen, short peptides containing S-antigen sequences were also tested. Among six known peptides, only one, with the sequence of GVDVE-VKAFATDITDAEED, was found to be mildly active (7.45 ± 1.20 nanomoles/10^6 cells).

AG, a specific inhibitor for iNOS, exhibited nearly total suppression (95% for S-antigen and 94% for IRBP) in RAW 264.7 macrophages, whereas the same concentration of AG itself produced no appreciable effect on endogenous nitrite production (Fig. 4). To test the tyrosine kinase dependence of S-antigen activation, the effect of genistein, a tyrosine kinase inhibitor, was also evaluated. With 100 μM genistein, the suppression of nitrite production in S-antigen was nearly 90% (Fig. 4). To evaluate the possible contamination of LPS in our protein preparations, polymyxin B, an inhibitor for LPS activation, was coincubated with the retinal proteins. Suppression of LPS-mediated activation (100 ng/ml) by polymyxin B was found to be nearly total; at the same time, the inhibition obtained for retinal proteins was 10% and 12% for S-antigen and IRBP, respectively (Table 1). With the lower concentrations of S-antigen and IRBP (10 μg/ml), the effect of polymyxin B (10 μg/ml) was negligible. In S-antigen, the nitrite production with and without polymyxin B was 7.19 ± 1.21 and 7.15 ± 1.30 nanomoles/10^6 RAW 264.7 cells, respectively, whereas in IRBP, these numbers were 5.84 ± 0.81 and 5.92 ± 1.10 nanomoles/10^6 RAW 264.7 cells, respectively.

For evaluating the combined effects of antigens, the combination of S-antigen (25 μg/ml) and IRBP (25 μg/ml) was first attempted, and then S-antigen and IRBP (each 25 μg/ml) were further combined separately with 10 U/ml IFNγ, 25 ng/ml LPS, and 500 U/ml TNFα. In all these combined experiments, the effects were all additive and not synergistic (Fig. 5).

**NF-κB Activation**

Because the induction of iNOS by cytokine has been shown to involve the activation of nuclear transcription factor NF-κB, we examined whether S-antigen and IRBP induced NF-κB activity in RAW 264.7 cells. S-antigen induced a time-dependent increase in NF-κB activity during a period of 30 to 120 minutes (Fig. 6). Addition of excess competing oligonucleotide reduced NF-κB activity by 90%, indicating specific incorporation in the NF-κB band. Treatment of RAW 264.7 cells with 5 μM PDTC (an NF-κB inhibitor), 5 μg/ml herbimycin A (a tyrosine kinase inhibitor), 10 μM PD98059 (an MAP kinase inhibitor), and 20 nM GF 109203 X (a protein kinase C inhibitor) abrogated S-antigen–induced activation of NF-κB.

**Superoxide Production**

With primary cultures of rabbit peritoneal macrophages, 50 μg/ml S-antigen induced superoxide production of 13.2 nanomoles/10^6 cells, which was approximately 70% of that produced by 0.5 μM fMLP, a potent stimulator for superoxide production in these systems (Fig. 7). Under the same conditions, 50 μg/ml IRBP did not induce superoxide production, and MBP produced a low level of superoxide, amounting to approximately 50% of that produced by S-antigen. The kinetics of production stimulated by S-antigen were similar to those exhibited by fMLP and other potent stimulators (Fig. 8). Superoxide production began immediately without an appreciable lag period and was nearly linear for up to 30 minutes, after which it exhibited a plateau, and increase was minimal. RAW 264.7 produced no measurable amounts of superoxide, and rat peritoneal macrophages generated minimal amounts (2.0 nanomoles/10^6 cells) of superoxide after stimulation by either fMLP or S-antigen.

**DISCUSSION**

In the present study, S-antigen and IRBP, the uveitogenic soluble proteins concentrated in the photoreceptors, were found to induce NO production in the RAW 264.7 macrophage cell line and in rat peritoneal macrophages. This stimulatory effect is not due to contamination of retinal proteins by endotoxin, because the stimulatory activity was not significantly affected by the addition of polymyxin B, a known inhibitor of endotoxin. That the stimulatory effect of bovine S-antigen and IRBP was not due to the xenogenic effect on the tested macrophages was indicated by the observation that the retinal soluble proteins from rat also displayed the same effect on rat macrophages. The other uveitogenic proteins from retina, such as rhodopsin, recoverin, and phosducin, had no stimulatory effect. With both S-antigen and IRBP, there was a lag period of 6 hours, after which the amount of nitrite increased in a linear fashion for up to 48 hours. Presumably, the observed lag period was required for transcription and translation of iNOS. The generation of NO by both S-antigen and IRBP was also dose dependent up to 50 μg/ml. Above this concentration, it reached a plateau, possibly indicating a saturable receptor, and the induction was totally inhibited by 5 mM AG, a specific inhibitor of iNOS. No synergistic effect was found when S-antigen and IRBP was combined with IFNγ, LPS, or TNFα. Incubation of S-antigen with rabbit peritoneal macrophages also induced the production of superoxide, although the amount of superoxide produced was less than that observed with the potent stimulator fMLP.

S-antigen is a soluble protein of 50 kDa, ubiquitously found in the retina, but its immunoreactivity is concentrated in photoreceptors. The normal function of S-antigen is believed to be restoration of the visual cycle after excitation by blocking the binding between rhodopsin and transducin. Interphotoreceptor retinoid-binding protein facilitates the transport of retinol from pigment epithelium to retinal photoreceptors. Both S-antigen and IRBP are effective agents used widely for inducing EAU in animals. However, the role of S-antigen or
IRBP in inducing the formation of reactive oxygen metabolites or nitrogen metabolites has not been investigated.

AG, a nucleophilic hydrazine compound, has recently been identified as a first selective inhibitor of iNOS.20 In vitro, AG has been demonstrated to suppress the effect of LPS- and cytokine-induced nitrite formation.24 In the present study, using a relatively low concentration of AG, we also observed the inhibition of nitrite formation by S-antigen. In S-antigen stimulation, the accumulation of nitrite was inhibited by pretreatment with genistein, a specific inhibitor of tyrosine kinase, suggesting that the S-antigen–induced activation involves tyrosine kinase. S-antigen may initially bind to receptors on the macrophage cell membranes, triggering the ligand-dependent tyrosine kinase activity and causing the elevation of second messengers, such as Ca\(^{2+}\) and IP\(_3\).25,26 which could lead to the activation of transcription factor NF-κB. This is supported by our results showing S-antigen caused increased NF-κB activity, which was inhibited by a tyrosine kinase inhibitor. Studies have shown that the murine promoter region of iNOS contains at least 24 consensus sequences for the binding of transcription factors. Among these, the NF-κB family of proteins appear to be essential components for the transactivation of iNOS.27 Our results show that PDTC, a specific inhibitor of NF-κB, blocked S-antigen–induced activation of NF-κB in RAW 264.7 cells, thus indicating that S-antigen–induced formation of NO occurred as a result of NF-κB activation.

S-antigen also displayed the capability to stimulate peritoneal macrophages to produce superoxide, similar to a number of other agents. This is shown in Figure 5, where S-antigen and IRBP were combined with other agents to examine their effects on nitrite production.

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932899/)  
**Figure 5.** Combination effect of S-antigen and IRBP with other agents. Using RAW 264.7 cells (1 × 10^6 cells), S-antigen (25 μg/ml) was combined with IRBP (25 μg/ml), IFNγ (10 U/ml), LPS (10 ng/ml), or TNFα (500 U/ml) and were then incubated for 48 hours at 37°C. These agents were also combined with IRBP (25 μg/ml). The nitrite production was estimated by the Griess reaction. Data are means ± SD for three determinations. S-Ag + IRBP: S-antigen (25 μg/ml) + IRBP (25 μg/ml); S-Ag + IFN: S-antigen (25 μg/ml) + IFNγ (10 U/ml); S-Ag + LPS: S-antigen (25 μg/ml) + LPS (10 ng/ml); S-Ag + TNF: S-antigen (25 μg/ml) + TNFα (500 U/ml); IRBP + IFN: IRBP (25 μg/ml) + IFNγ (10 U/ml); IRBP + LPS: IRBP (25 μg/ml) + LPS (10 ng/ml); IRBP + TNF: IRBP (25 μg/ml) + TNFα (500 U/ml).

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**Figure 6.** Effect of inhibitors on S-antigen-induced NF-κB activation. RAW 264.7 cells (1 × 10^6 cells) were incubated with 50 μg/ml S-antigen in the presence and absence of PDTC (5 μM), herbimycin A (5 μg/ml), PD 98059 (10 μM), and GF 109203X (20 nM) for the indicated time. Nuclear extracts were prepared for the determination of NF-κB activity by electrophoretic mobility-shift assay. The experiments were repeated twice, and representative results are shown.
of molecules, such as C5a, interleukin-8, plasmalogens, platelet-activating factor, and fatty acids. Recently, both a vasoactive intestinal peptide with 28 amino acids and a neuromodulatory peptide of 36 amino acids have been found to stimulate peritoneal macrophages to produce superoxide anion. Superoxide formation by these peptides appears to be facilitated by the presence of receptors for these neuropeptides in peritoneal macrophages, and their effect presumably occurs as a result of protein kinase C activation. An undecapeptide, substance P, which is present in the mammalian nervous system, activates reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase by binding to a receptor distinct from the fMLP receptor.

Intraocular inflammation, such as uveitis, is characterized by a rapid infiltration of polymorphonuclear leukocytes into the retina, choroid, and anterior segments. This is then followed by a slower, but longer lasting infiltration of macrophages. Under these conditions, activated phagocytes simultaneously release high levels of NO and superoxide. Although the chemical reactivity of both NO and superoxide is low, these two can combine rapidly to form the much more potent oxidant peroxynitrite. Photoreceptors are especially prone to the attack by peroxynitrite, because of the high content of docosahexaenoic acid (22:6). Lipid peroxidation of photoreceptors and nitration of retinal proteins by peroxynitrite have recently been demonstrated in our laboratory.

These reports and the present study suggest that in EAU, the retinal soluble proteins not only function as autoantigens, but they may also enhance the inflammation and retinal damage by inducing NO and superoxide generation. This finding could provide a basis for the marked damage noted in the outer retina in EAU. Such observations also suggest that in humans with severe uveitis, retinal degeneration could be enhanced by the soluble retinal proteins. However, additional in vivo studies on EAU are required to investigate this autodestructive process induced by the retinal proteins, particularly in inflammation-mediated retinal degeneration.

In this study, we have demonstrated that the retinal soluble proteins S-antigen and IRBP, which are abundantly present in photoreceptors, are capable of stimulating nonprimed macrophages to produce substantial amounts of reactive nitrogen and oxygen metabolites. The effect displayed by S-antigen in the production of nitrogen metabolite appears to be regulated by the sequence of S-antigen and, therefore, the feasibility of receptor occupancy as an initial event. S-antigen has been shown to occupy the receptor for TNFα and induces TNFα production in monocytes. Inflammation, such as in uveoretinitis, the effect of these retinal proteins may function either additively or synergistically in enhancing inflammation-mediated tissue damage.

References